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METAGENOMICS OF PREBIOTIC AND PROBIOTIC SUPPLEMENTED BROILERS GASTROINTESTINAL TRACT MICROBIOME

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ABSTRACT

Phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) is a recently developed computational approach for prediction of metagenomics , comparing marker gene data with a reference genome database. In the current study, we used PICRUSt for predicting metagenomics in broilers subjected to heat stress, and supplemented with prebiotic and probiotic. Cecal digesta were taken for DNA extraction. DNA was sequenced using 16S rRNA pyrosequencing. Sequences were analyzed using Qiime and PICRUSt to predict metagenomics. Functional genes content inference was consigned according to Kyoto Encyclopedia of Genes and Genomics Orthology Hierarchy and compared using Linear Discriminant Analysis and the Kruskal-Wallis test. The core gene contents of broilers gut microbiome were predominantly associated with metabolism (52.3%) and environmental information processing (27.4%). Among metabolic processes, carbohydrates metabolism (20.0%) was highest, followed by xenobiotics (11.0%), amino acids (7.9%), and lipids metabolism (3.3%). The information processing gene included; membrane transportation (21.0%), signal transduction (3.1%), and signaling molecules interaction (3.2%). Other significant pathways identified in the broilers gut microbiome are genetic information (7.6%), cellular (0.86%), and organismal (0.21%) processes. About 12.3% genome in this study was unclassified. Among different treatment groups, genetic information processing was higher ($P<0.05$) in the probiotic supplemented group compared to all the other groups. However, no significant differences ($P>0.05$) were observed for other metabolic and cellular processes. In conclusion, the present study reveals that gut microbiome of broilers significantly contributes to the host metabolism and nutrients absorption, and the stress and supplements have no significant effects to change these functions.

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INTRODUCTION

Gastrointestinal tract (GIT) hosts a diverse, dynamic, and gigantic microbial community, mainly dominated by bacteria, protozoa, viruses, fungi, and archea. This microbial community, commonly referred to as microbiome, plays significant role in modulating host's immune system, body homeostasis, and physiology [1]. Recent advances in high throughput sequencing techniques have facilitated our understanding of microbial ecology, gleaned with the information about its physiological functions. Metagenomics, study of whole genome of a community, uses modern sequencing platforms which can generate more than 1 gigabyte data in a single run. Perhaps, profiling functional genome at relatively larger scale in routine is very expensive. Conversely, sequencing of 16S ribosomal RNA gene (16S) for bacterial and archaeal phylogeny and taxonomic characterization offers a cheap alternative, with certain limitations. The 16S sequencing uses less depth to describe microbial diversity [2] whereas, deep and more costly metagenomic sequencing offers complete information about phylogeny and functional gene contents of a community [3].

The 16S sequencing and metagenomics provide two different types of the information, which are strongly, though imperfectly, correlated [4]. The association is strongly related with core and pan genomics of the phylogenetic clades, comprising genome of all members of a clade. In another study [5] have correlated 16S based phylogeny and the functional attributes of several gut and soil microbiomes, using a computational approach, phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt). Though, the precision of this method has not been assessed, but the association between genomics and phylogeny, as attributed by several studies [4, 6-8] advocates that it is conceivable to approximately predict the functional attributes of microbiome from phylogeny.

In the present study, we used 16S data from a broiler study conducted at United States Department of Agriculture, USA. The study included four hundred and fifty d old chickens, randomly divided in five treatment groups. The objective of the study was to elucidate effect of supplementation of probiotic and prebiotic on growth performance and gut microbial community of broilers kept under heat stress conditions.

MATERIALS AND METHODS

Four hundred and fifty d old chicenks were randomly divided in five treatment groups as described earlier [9]. On d 42, five birds per group were killed by CO_2 asphyxiation. Cecal digesta were collected and subjected to DNA extraction, following manufacturer's prescribed protocol (QIAgen Corporation, Valencia, CA). The experimental protocol and animal husbandry met or exceeded guidelines set forth by the USDA, ARS, SPARC, FFSRU Animal Care and Use Committee Experimental Animal Protocol. Animal health and well-being were continuously monitored by a FFSRU staff veterinarian.

16S rRNA throughput sequencing

Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) of 16S rRNA (V1–V3 region was performed using Gray28F 5'TTGATCNTGGCTCAG and Gray519R 5'GTNTTACNGCGGCKGCTG primers [10]. The pyrosequencing was performed at Research and Testing Laboratory (Lubbock, TX), following their standard procedure. The raw sequence data was trimmed, denoised, and chimera depleted before passing it through QIIME (QIIME V 1.7). The .FNA format obtained from 454-Roche platform was used to pick closed reference operational taxonomical units (OTUs) against green genes 13_5_OTUs. The experimental OTUs were normalized. Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used to infer the approximate gene content of the detected 97% OTUs in our dataset using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) (v0.1) [11, 12].

Statistical Analysis

To test the normal distribution in the data the Kolmogorov-Smirnov test was applied. The Kruskal–Wallis test was used to find difference (P<0.05) in median percentage values of the predicted function (SPSS 13.0; Chicago, IL). Linear discriminant analysis (LDA) of relative abundances of all predicted functions between different treatment groups was determined using at an alpha value of 0.05 for the pairwise Wilcoxon test between subclasses at threshold of 2.0 on the logarithmic LDA score for discriminative features.

RESULTS

Table 1 presents median percentages of cellular and metabolic processes analyzed using Kruskal–Wallis test. The core metagenomics of cecal microbiome of broilers reveals 52.3% gene contents corresponded to different metabolic processes, including carbohydrates (20.0%), xenobiotics (11.0), amino acids (7.9%), lipids (3.3%), and energy metabolism (2.8%). Theenvironmental information processing gene (27.4%) contents included; membrane transportation (21.0%), signal transduction (3.1%), and signaling molecules interaction (3.2%). Among different treatment groups, no significant differences were observed on metabolism and environmental information processing gene contents. The genetic information processing gene contents were higher (P< 0.05) in the probiotic supplemented group compared to all the other treatment groups. Cellular processes, which included cell growth, mortality, and catabolism gene contents, only accounted 0.86% of the total microbiome genome. About 12.3% genome observed was unclassified into appropriate categories. The LDA of relative abundances of all predicted metagenome between different treatment groups revealed no significant effects.

DISCUSSION

In the recent era of technology advancement, the invention of next-generation sequencing platforms and computation tools has tremendously enhanced our understanding of complex consortium between the host and the microbes living inside their gut. To understand microbial diversity and their functional association with the hosts, this study was performed which utilized four hundred

and fifty broilers. The review of literature reveals that this is earliest of its kind of studies that utilized poultry broilers to study gut metagenomics. The high throughput 16S sequencing of cecaldigesta yielded 174,440 sequences, belonging to different bacterial clades.

About 103 metabolic pathways were identified, which stayed consistently similar among different treatment groups. The metagenomics revealed that most microbial genome corresponded to carbohydrate, xenobiotic, amino acid, and lipids metabolism. These findings are partially in agreement with the work of Danzeisen, Kim, Isaacson, Tu and Johnson [13], who reported a high metabolic potential in cecum microbiome of chickens fed anticoccidia and growth promoter agents. Similarly, Waite and Taylor [14] reported that in captive birds predictive metagenome was mainly associated with carbohydrate metabolism, followed by amino acids metabolism, disease inducing genome, and cell signaling. Further, diets had no effect on metabolism. In comparison to the present findings, earlier studies have reported that poultry fed probiotic and prebiotic have higher metabolic rates [15].

Table 1. Metagenomic functional prediction for broilers cecal microbiome.

KeggOrtholog	*Median% Thermo neutral	HS-Control	MOS	PM	SYN	Standard Deviation	Kruskal Wallace P-value
Cellular Processes	**0.98**	**0.89**	**0.73**	**0.64**	**1.04**	**0.18**	**0.07**
Cell Growth and Death	0.00	0.42	0.28	0.20	0.56	0.02	0.09
Cell Motility	1.05	0.98	0.84	0.97	0.98	0.56	0.16
Transport and Catabolism	1.88	1.29	1.08	0.75	1.58	0.24	0.58
Environmental Information Processing	**26.92**	**27.62**	**25.86**	**25.83**	**30.89**	**1.79**	**0.05**
Membrane Transport	21.89	20.25	22.45	18.04	22.50	0.19	0.06
Signal Transduction	2.51	3.08	2.29	3.78	4.14	1.41	0.05
Signaling Molecules and Interaction	2.52	4.29	1.12	4.01	4.25	0.20	0.08
Genetic Information Processing	**7.72^{b}**	**7.25^{b}**	**6.17^{b}**	**10.75^{a}**	**6.18^{b}**	**0.59**	**0.04**
Folding, Sorting and Degradation	1.28	0.78	0.56	2.52	1.37	0.36	0.06
Replication and Repair	2.42	3.81	2.54	4.01	2.74	0.00	0.09
Transcription	2.38	2.21	2.11	2.72	1.86	0.82	0.15
Translation	1.64	0.45	0.96	1.50	0.21	0.87	0.28
Metabolism	**54.85**	**48.91**	**51.36**	**51.38**	**54.81**	**3.07**	**0.08**
Amino Acid Metabolism	8.10	7.18	8.58	8.18	7.44	2.21	0.18
Biosynthesis of Other Secondary Metabolites	0.30	0.01	0.45	0.05	0.00	0.90	0.15
Carbohydrate Metabolism	20.50	19.24	19.17	19.34	21.59	3.51	0.58
Energy Metabolism	3.19	2.58	2.08	2.63	3.41	0.21	0.71
Enzyme Families	0.30	0.11	0.79	0.16	0.72	0.00	0.17
Glycan Biosynthesis and Metabolism	0.75	0.61	0.29	0.67	0.02	0.00	0.09
Lipid Metabolism	3.56	3.42	3.88	3.42	2.19	0.29	0.21
Metabolism of Cofactors and Vitamins	2.24	2.19	2.02	2.32	2.95	0.18	0.48
Metabolism of Other Amino Acids	0.92	0.22	1.52	1.54	1.14	0.08	0.29
Metabolism of Terpenoids and Polyketides	2.60	2.35	2.49	2.49	2.65	0.14	0.49
Nucleotide Metabolism	0.61	0.25	0.01	0.28	0.01	3.58	0.27
Xenobiotics Biodegradation and Metabolism	11.60	10.25	10.08	10.30	12.69	2.78	0.28
Organismal Systems	**0.00**	**0.60**	**0.00**	**0.62**	**0.00**	**0.01**	**0.07**
Endocrine System	0.00	0.60	0.00	0.62	0.00	0.00	0.18
Unclassified	**12.26**	**12.96**	**12.34**	**12.05**	**11.81**	**0.14**	**0.24**
Cellular Processes and Signaling	3.72	4.08	3.55	3.12	3.61	0.00	0.42
Genetic Information Processing	0.61	0.49	0.12	0.58	0.45	0.24	0.08
Metabolism	4.36	4.66	4.85	4.38	4.28	0.29	0.07
Poorly Characterized	3.58	3.73	3.82	3.94	3.47	1.07	0.11

*Median relative gene pathway abundance of significantly abundant modules (Kruskal Wallace $P \leq 0.05$) for Thermoneutral, Heat stressed Control, Mannan-oligosaccharides, probiotic mixture, and Synbiotic supplemented groups.
Also, it is known that poultry kept under high temperature has poor metabolism [16].

Genetic and environmental information processing genome was also abundantly present in all treatment groups. This class includes a range of genes associated with membrane transport, cell signal transduction, translation, cell adhesion, and cytokine receptors and is probable involved in host/bacteria interactions. Among different treatment groups, probiotic fed group had significantly higher percentage of these genes, depicting that probiotic supplementations can enhance these processes in microbes and boost host immunity [17]. These findings are supported by several studies [18, 19]. In the current experiment only 12.28% genome is unclassified with no clear genetic map.

In conclusion, this study used computational bioinformatic tool to study functional genome contents of broilers cecal microbiome. The metagenomics prediction tool, PICRUSt, provides an important insight into the broilers cecal milieu and refers promotion of digestion and transportation of the nutrients in the broilers ceca. Further, this study reveals that stress and supplementation of probiotic and prebiotic have no effect on microbiome functioning genome.

AUTHORS' STATEMENTS

Authors hereby declared that the contents of the present study are product of their own research and no part has been copied from any published source.

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